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Survey on relationship between acute phase proteins (serum amyloid A, milk amyloid A and serum haptoglobin) in inflammatory diseases of dairy cattle

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Abstract

The objective of this study was to evaluate the concentration of some acute phase proteins (serum amyloid A, milk amyloid A and serum haptoglobin) and alteration in hematological values in some inflammatory diseases in dairy cattle. Fifty-six *Holstein* dairy cows with inflammatory diseases were used in this study including: subclinical mastitis, hematological infections (theileriosis and anaplasmosis), metritis, pneumonia and displacement of abomasum (n= 8 each). Clinically healthy cows were selected as control group (n= 8). Blood samples were collected from jugular vein of both groups. Milk samples were taken from four quarters of each cow and mixed together and then one sample of pooled milk from both groups was taken. There were significant differences in mean concentrations of serum and milk amyloid A between clinically healthy cows and diseased cows and also, between abomasal displacement and other infectious diseases ($p<0.05$). There were also significant differences in haptoglobin concentrations between cattle suffering from subclinical mastitis, theileriosis and pneumonia compared to those with anaplasmosis, abomasal displacement and metritis ($p<0.05$). Our results indicated the application of serum haptoglobin, serum amyloid A and milk amyloid A measurements as indicators of inflammatory diseases in dairy cattle. In conclusion, the current study shows that haptoglobin, serum amyloid A, and milk amyloid A measurements, could be successfully applied in cattle as valuable indicators of inflammatory diseases.

Keywords: Acute phase protein, serum amyloid A, haptoglubin, inflammatory diseases

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Introduction

Detailed functions of the acute phase proteins (APPs) are not fully understood, however, it is suggested that they are mainly engaged in processes like opsonization and trapping of microorganisms and their products, binding of cellular remnants (Whicher and Westacott, 1992), complement activation, neutralization of enzymes, and scavenging of free radicals and hemoglobin (Niewold *et al.*, 2003). In the recent studies concerning new indicators of mastitis, acute phase proteins are strongly implicated (Eckrsall *et al.*, 2001 Grönlund *et al.*,2003, Nielsen *et al.*,2004, Grönlund *et al.*,2005, , O´Mahony *et al.*, 2006, Ihnat *et al.*, 2007, Akerstedt *et al.*,2007, , Hiss *et al.*, 2007). APPs are often determined in serum of animals to monitor their health (Kostro *et al.*,2001; Murata *et al.*, 2004, Petersen *et al.*, 2004). APPs can be valuable to the veterinarian in identification of cows with inflammatory diseases and the stages of disease can be better evaluated by monitoring more than one APP like serum amyloid A (SAA) and serum haptoglobin (Hp), so chronic conditions as well as acute ones should be evaluated and characterized by APP profiling (Karreman *et al.*, 2000, Eckersall, 2004). Hp is an α_2-globulin and it is one of the APPs, which increases in serum in acute inflammatory diseases. Measurement of APPs could be a useful tool for evaluation of health in calf herds (Ganheim *et al.*, 2007). SAA is an apolipoprotein of high-density lipoprotein (Nakayama *et al.*,1993, Gruys *et al.*,1994; Husby *et al.*,1994). It is described to bind lipopolysaccharide, comparable to lipopolysaccharide binding protein (Schroedl *et al.*, 2001). There are no published reports about the comparison of serum Hp, SAA and MAA concentrations in subclinical mastitis, theileriosis, anaplasmosis, pneumonia, metritis, and abomasal displacement. Therefore, the aim of this study was to evaluate these APPs as inflammatory indicators in various inflammatory diseases of cows.

Materials and methods

Fifty-six *Holstein* cows with various inflammatory diseases from three dairy farms located in Tabriz suburb were included in this research. Eight clinically healthy adult cattle were also selected randomly as control. The diseased cows used in this study included subclinical mastitis, parasitic infections (theileriosis and anaplasmosis), pneumonia, metritis and abomasal displacement (n=8 each). The presence of disease was assessed on the basis of clinical examination and laboratory findings. Diseased cows were thoroughly examined and blood samples were collected from jugular vein of both groups for hematology, clinical biochemistry and other relevant analysis. To determine serum Hp, SAA and MAA serum was separated by centrifugation at 750 g for 15min. Serum samples were stored at -20 °C until analysis. SAA and MAA concentrations were measured by using a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) (Tridelta Development Pic, Co.Wicklow, Ireland) and Hp was measured on the basis of the preservation of the peroxidase activity of hemoglobin which is directly proportional to the amount of haptoglobin using a colorimetric commercial kit (Tridelta Development Plc, Co., Wicklow, Ireland). Statistical analysis was performed using SPSS software (Ver. 16, IBM Corporation, USA).

The data were checked for errors and compared with written reports; Outliers were rechecked during data processing to ensure that values were accurate. All variables were screened for normality by visual assessment of the distributions. All values were expressed as mean and standard error (mean±SE). Differences between groups were analyzed using one way ANOVA and Duncan's multiple comparison tests and considered as statistically significant when the *p* value was less than 0.05.

Results

The mean concentrations of SAA and MAA and their correlation in cows with

inflammatory diseases and clinically healthy cows are presented in Table 1. Statistical evaluations showed that there was no significant correlation between SAA and MAA in each of inflammatory diseases ($p<0.05$). There were significant differences in mean concentrations of SAA and MAA between clinically healthy cows and diseased cows and also, between abomasal displacement and other infectious diseases ($p<0.05$). Significant difference in Serum Hp concentrations was observed between clinically healthy cows and those with inflammatory diseases ($p<0.05$).There were also significant differences in Hp concentrations between cattle suffering from subclinical mastitis, theileriosis and pneumonia compared to those with anaplasmosis, abomasal displacement and metritis ($p<0.05$).

Table 1. SAA and MAA concentration (mean ± SE) in cows with inflammatory diseases and clinically healthy cows.

Groups / Parameters	Number	SAA (μg/ml)	MAA (μg/ml)	Correlation coefficient
Subclinical mastitis	8	29± 2.1[a]	15 ± 0.9[a]	-0.03
Theileriosis	8	33± 3.9[a]	29 ± 1.2[b]	-0.0033
Anaplasmosis	8	30± 4.3[a]	17 ± 2.6[b]	-0.003
Pneumonia	8	36± 2.6[a]	28± 3.8[b]	-0.0039
Metritis	8	41± 0.09[a]	61 ± 1.1[c]	0.008
Abomasal displacement	8	19± 4.9[b]	8± 0.8[d]	-0.038
Clinically healthy cows	8	6 ± 0.5[c]	0.5± 0.08[e]	0.04*

Means within a column with different superscript letters (a, b, c, d, e) significantly differ ($p<0.05$).
*Indicates $p<0.05$

Discussion

Numerous studies have described the serum concentration of APPs in animals with various diseases, but only a few are devoted to their levels in milk from cows with inflammatory disease. Therefore, the present study was undertaken to determine the concentration of SAA, MAA and serum hptoglobin and assess their usefulness for the detection of common inflammatory diseases in cows. Quantification of APP concentration in serum can provide valuable diagnostic information in the detection, prognosis, and monitoring of diseases in several animal species. The inclusion of APPs measurements in health monitoring programs on a herd basis in livestock has been suggested, not only for the identification of individual animals with disease, but also as a mean of identifying animals with subclinical disease. Measurement of APPs, especially Hp and SAA, as indicators of health in herds has gained an increasing interest among researchers (Murata *et al*., 2004, Ganheim *et al*., 2007). Hp and SAA have been found to increase in the serum of cattle affected by many different diseases (Alsemgeest *et al*.,1994, Horadagoda *et al*.,1994, Godson *et al*.,1996, Hirvonen *et al*.,1996, Smith *et al*.,1998, Heegard *et al*., 2000, Sheldon *et al*.,2001, Nielson *et al*., 2004, Murata *et al*.,2004, Grounland *et al*., 2005, Fe´lix *et al*., 2008). SAA could be a useful diagnostic marker of early post-operative complications and also for evaluation of health in calves (Dabrowski *et al*.,2007, Ganheim *et al*., 2007).There is only one published report about the comparison of SAA and MAA concentrations in some infectious diseases (Nazifi *et al*., 2008a). In the present study the comparative measurement of SAA and MAA as well as serum Hp was conducted as inflammatory indicators in various inflammatory diseases of cattle in East Azarbaijan province located in northwest of Iran. The previous study has revealed that the concentration of SAA was higher in serum and milk of the cows with mastitis than in the cows with extramammary inflammatory conditions (Nielsen *et al*., 2004). However, in the present study the mean concentrations of SAA and

MAA were lower in serum and milk of those with subclinical mastitis and abomasal displacement than in the cows with other inflammatory diseases. The reason may be that the severity of these two diseases is lower than other inflammatory diseases. The mean concentration of serum Hp in our study was also at the minimum value in cows with abomasal displacement than the others. SAA concentrations below the detection limit were considered as a good indicator of healthy udder quarters (Gronlund *et al*., 2005). The results of the present investigation showed that there is no correlation between concentration of SAA and MAA in each of the inflammatory diseases under study. These results can be due to intramammary synthesis and secretion of SAA3 to colostrum and mastitis milk and has beneficial functions for the gut mucosa of the offspring (Eckersall *et al*., 2001, McDonald *et al*., 2001, Mack *et al*., 2003, Larson *et al*., 2003a,b, Larson *et al*., 2005).In diseased cattle, the lowest conce ntrations of Hp (0.075±0.018 mg/mL) were observed in cows with abomasal displacement (Table 2). In the present study, the concentration of SAA and Hp in cows infected with *Theileria annulata* was significantly higher than the clinically healthy cows (p<0.05). An effect of free hemoglobin in serum samples towards reduction of measured concentration of Hp has been found (Eckersall *et al*., 1999, Petersen *et al*.,2001). These data are different from our results in cattle with anaplasmosis and theileriosis, in which we observed increased serum Hp concentrations. In one study reported by Glass *et al*. (2003), following experimental infection with *Theileria annulata*in in cattle, Hp appeared only in some of the animals, and generally at a low level. This shows that Hp should be used with caution as a marker of inflammation in Theileriosis. In the present study, the concentration of SAA and serum Hp in cows with pneumonia and metritis was significantly higher than clinically healthy cows (p<0.05). Alsemgeest *et al*. (1994) introduced Hp as a valuable marker for differentiating healthy cows from pneumonic ones. Godson *et al*. (1996) evaluated APPs in cows with respiratory infections and introduced Hp as a diagnostic factor. Berry *et al*.(2004) found that Hp concentrations were higher in calves treated by respiratory disease for multiple times compared with those never treated, or treated on a single occasion. Increasing in Hp and SAA amounts has been reported after intratracheal inoculation with *Manheimia hemolytica* (Horadagoda *et al*., 1994, Cheryk *et al*., 1998). In diseased cows subjected to our study (n=48), Hp levels were significantly higher (0.132±0.069 mg/mL) than those in healthy ones (0.02±0.14 mg/mL; p<0.05). Increased serum Hp concentration in cattle was found in various acute infections under field conditions (Skinner *et al*., 1991, Alsemgeest *et al*., 1994), mastitis (Hirvonen *et al*., 1999, Ohtsuka *et al*., 2001, Gronlund *et al*., 2003, Nielsen *et al*., 2004, Gronlund *et al*., 2005), and metritis (Smith *et al*., 1998). Our study showed that acute respiratory infections resulted in a significant increase of serum Hp levels. These results are in agreement with one previous study carried out by Nazifi *et al*. (2008b). Similar findings were demonstrated in cattle with experimental infection with *Pasteurella haemolytica* (Katoh & Nakagawa, 1999, Ganheim *et al*.,2003).In our study, subclinical mastitis, metritis, and abomasal displacement were also found to provoke significantly elevated Hp values.

In conclusion, the current study shows that Hp, SAA, and MAA measurements could be successfully applied in cattle as valuable indicators of inflammatory diseases. The studied parameter could be influenced by other disorders and significant changes in hemoglobin levels; so it should not be used independently but together with other blood laboratory indices with regard to better elucidation of the systemic status. However, APPs increase during the development of the disease and decrease in the recovery stage could be used for diagnosis of the disease in early stages.

Table 2. Serum Hp concentrations (mean ± SE) in cows with inflammatory diseases and clinically healthy cows.

Groups / Parameters	Number	Serum Hp (mg/ml)
Subclinical mastitis	8	0.15± 0.09a
Theileriosis	8	0.18± 0.08a
Anaplasmosis	8	0.08± 0.12b
Pneumonia	8	0.22± 0.03a
Metritis	8	0.09± 0.08b
Abomasal displacement	8	0.075 ± 0.018b
Clinically healthy cows	8	0.02 ± 0.14c

Means within a column with different superscript letters (a, b, c, d) significantly different(p<0.05).

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بررسی ارتباط بین پروتئین‌های فاز حاد انتخابی (آمیلوئید سرمی و شیری A و هاپتوگلوبین) در بیماری‌های التهابی گاوهای شیری

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چکیده

هدف از مطالعه حاضر بررسی غلظت سرمی پروتئین‌های فاز حاد انتخابی (آمیلوئید سرمی و شیری A و هاپتوگلوبین) و نیز تغییرات بوجود آمده در پارامترهای خونشناختی برخی از بیماری‌های التهابی در گاوهای شیری نژاد هولشتاین است. ۵۶ راس گاو شیری هولشتاین که مبتلا به بیماری‌های التهابی مختلف از جمله ورم پستان تحت بالینی، عفونت های انگلی خون (تیلریوز و آناپلاسموز)، متریت، پنومونی و جابجایی شیردان بودند در این مطالعه انتخاب شدند (از هر بیماری ۸ راس گاو). تعداد ۸ راس گاو نیز به عنوان گروه کنترل در نظر گرفته شدند. سپس اقدام به خونگیری از ورید گردنی گاوهای مبتلا و سالم گردید. نمونه‌های شیر اخذ شده از چهار کارتیه پستانی هر دو گروه به‌طور جداگانه با هم مخلوط شدند و پس از آن اقدام به انجام آزمایش روی نمونه واحد شیری گردید. نتایج نشان داد که در رابطه با غلظت سرمی و شیری آمیلوئید A بین دو گروه گاوهای مبتلا و سالم و نیز بین جابجایی شیردان و سایر بیماری های عفونی اختلاف معنی‌داری وجود دارد ($p<0.05$). همچنین افزایش قابل ملاحظه‌ای در غلظت سرمی هاپتوگلوبین در گاوهای مبتلا به ورم پستان تحت بالینی، تیلریوز و پنومونی در مقایسه با گاوهای مبتلا به آناپلاسموز، جابجایی شیردان و متریت مشاهده شد ($p<0.05$). نتایج مطالعه حاضر کاربرد اندازه‌گیری پروتئین‌های فاز حاد انتخابی (آمیلوئید سرمی و شیری A و هاپتوگلوبین) به عنوان شاخص‌های ارزشمند التهابی را نشان می‌دهد و پیشنهاد می‌کند که این نشانگرها می‌توانند در شناسایی بیماری‌های التهابی موثر واقع شوند.

واژگان کلیدی: پروتئین فاز حاد، سرم آمیلوئید A، هاپتوگلوبین، بیماری‌های التهابی